DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/21/2010 has been entered.

Amended claims 1-12, 14, 18 and 21 are pending in the present application; and they are examined on the merits herein.

Priority

The present application is a 371 of PCT/EP04/11287, filed on 10/08/2004, which claims benefit of the provisional application 60/509,942, filed on 10/10/2003, and the foreign application EPO 03022780.5, filed on 10/10/2003.

Upon review of the specifications of the provisional application 60/509,942 and the foreign application EPO 03022780.5, and comparison with the specification of the present application, it is determined that the instant claims are only entitled **to the effective filing date of 10/08/2004** because both the provisional application 60/077,262 and the foreign application EPO 03022780.5 do not have a written support for the concept of cultivating chondrocytes at a first unphysiologically high extracellular concentration of magnesium (Mg), then cultivating the chondrocytes at a second unphysiologically high extracellular concentration of Mg, wherein said second

unphysiologically high extracellular concentration of Mg is increased over said first unphysiologically high extracellular concentration of Mg, and wherein said first unphysiologically high extracellular concentration of Mg is used during chondrocyte proliferation and said second unphysiologically high extracellular concentration is used during chondrocyte differentiation.

Response to Amendment

The rejections under 35 U.S.C. 103(a) that were set forth in the Office action mailed on 3/26/2010 were withdrawn in light of Applicant's amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-12, 14, 18 and 21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

An *in vitro* method for the generation of chondrons comprising the steps:

(a) cultivation of chondrocytes **in a monolayer culture** at a first unphysiologically high extracellular concentration of magnesium (Mg), then

(b) cultivating the chondoryctes of step (a) **in alginate gel** at a second unphysiologically high extracellular concentration of Mg, wherein said second

unphysiologically high extracellular concentration of Mg is increased over said first unphysiologically high extracellular concentration of Mg, and **wherein said first and second high extracellular concentrations of Mg range up to 20 mM**;

does not reasonably provide enablement for **other methods for the generation of chondrons as broadly claimed**. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. ***This is a new ground of rejection.***

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The instant specification is not enabled for a method for the generation of chondrons as broadly claimed for the following reasons.

1. The breadth of the claims

The claims are directed to a method for the generation of chondrons comprising the step of cultivation of chondrocytes at a first unphysiologically high extracellular concentration of magnesium (Mg), then cultivating said chondoryctes at a second unphysiologically high extracellular concentration of Mg, wherein said second unphysiologically high extracellular concentration of Mg is increased over said first

unphysiologically high extracellular concentration of Mg, **in both *in vitro* and *in vivo* and said first and second unphysiologically high extracellular concentrations of magnesium can be at any concentration in excess of 0.9 mM (e.g., 10 mM, 20 mM, 50 mM, 100 mM, 250 mM, 500 mM)**, and **said first unphysiologically high extracellular concentrarion of Mg is used during chondrocyte proliferation and said second unphysiologically high extracellular concentration is used during chondrocyte differentiation.**.

2. The state of the prior art and the unpredictability of the prior art

At the effective filing date of the present application (10/10/03), little was known on the effect of unphysiological high extracellular concentration of magnesium on chondrocytes during chondrocyte proliferation and differentiation phrases in the generation of chondrons as evidenced at least by the teachings of Egerbacher et al. (Vet Pathol 38:143-148, 2001; Cited previously), Valletta, G. (US 6,248,368; IDS), Garcia et al. (US 6,211,143; IDS), Halvorsen et al. (US 6,841,150; Cited previously), Jeschke et al (US 2002/0052044) and Masuda et al (US 001/0012965). Egerbacher et al disclosed that magnesium supplementation at 1X concentration (0.0612 mg/ml MgCl + 0.0488 mg/ml MgSO4 = 1mM MgCl + 0.4 mM MgSO4) or **at 3X concentration (about 4.2 mM Mg)** has a significantly positive effect on quinoline-treated horse and dog chondrocytes in 5-day monolayer cultures containing 10% FCS; and the positive effects of Mg supplementation include decreased cell loss and morphologic changes (outspread, stellate chondrocytes vs more spindle-shaped or spherical cells of quinolone-treated and Mg-free chondrocytes) and a slightly increased cell proliferation

(53% for Mg1 and 55% for Mg3) with respect to cells cultivated in Mg2+-free medium (47%; see page 146, col. 1, second paragraph; Figure 6). Valletta also disclosed a method for treating autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus and others, by administering orally **(dosage ranging 2 to 12 mg of magnesium per kg of body weight)** or parenterally (**dosage ranging from 2 to 30 mg/kg body weight daily)** a pharmaceutically acceptable composition suitable for releasing magnesium ions (e.g., organic or inorganic magnesium salts or complexes thereof) to a patient in need thereof (see at least the abstract; col. 3, line 31 continues to line 11 of col. 6; col. 5, lines 12-57). Even assuming that the maximal daily parenteral dosage of 30 mg magnesium/kg body weight is all available in blood, this dosage is only equivalent to about 15 mM magnesium assuming the molecular weight of magnesium is 24.3 g, blood is about 0.08% of body weight and 1 kg of blood is 1L). However, Valletta et al disclosed explicitly that **when blood magnesium levels are over 4 mM, a total loss of tendon reflex occurred, followed by myoparalysis, hypothermic coma and cardiac arrest** (col. 6, lines 3-6). Matsuda et al taught that amplification of chondrocytes or chondrogenic cells in alginate does not induce loss of the chondrocyte phenotype as occurs when amplification is performed in monolayer culture (paragraph 38); while Jeschke et al taught that in order to re-differentiate chondrocytes they are conventionally cultured in an alginate gel (paragraphs 15 and 17).

3. The amount of direction or guidance provided

Apart from the exemplifications showing the effect of magnesium sulfate on the proliferation of chondrocytes in a monolayer culture, and the effect of magnesium

sulfate on chondrocytes encapsulated in alginate beads during their re-differentiation phrase, wherein the maximal concentration of magnesium sulfate tested was 20 mM (examples 1-3), the instant specification fails to provide sufficient guidance for a skilled artisan on how generate chondrons by cultivating chondrocytes during proliferation and differentiation phrases in both *in vitro* and *in vivo* at unphysiologically high extracellular concentration of magnesium in excess of 20 mM. Even 3 years after the effective filing date of the present application (10/10/2003) Feyerabend et al (Tissue Engineering 12:3545-3556, 2006) still demonstrated that **unphysiologically high magnesium concentration supplementation has adverse effects on chondrogenesis by inhibiting extracellular matrix formation and reducing the effects of growth factors (see abstract and Figures 6-7), magnesium concentration at 20 mM already led to a significant inhibition of chondrocyte proliferation (Figure 1), with the highest magnesium concentration that was tested was 30 mM in a proliferation assay**. Moreover, Valletta et al already disclosed that when blood magnesium levels are over 4 mM, a total loss of tendon reflex occurred, followed by myoparalysis, hypothermic coma and cardiac arrest (col. 6, lines 3-6). The instant specification fails to provide any evidence indicating that any unphysiological high magnesium concentration **in excess of 20 mM** would be beneficial for the proliferation and/or differentiation of chondrocytes in vitro, let alone in vivo given known toxicities caused by unphysiologically high extracellular magnesium concentrations. Apart from culturing chondrocytes embedded in alginate gel for re-differentiation, the instant specification also fails to provide sufficient guidance on how to differentiate the already

differentiated chondrocytes under any other conditions in vitro in order to practice the method of generating chondrons as claimed broadly. Additionally, the instant specification also fails to provide any guidance for an ordinary skill artisan on how or when one knows that differentiation of already differentiated chondrocytes in vivo occurs so that .a second unphysiologically high extracellular concentration of Mg can be used. Furthermore, the effect of unphysiologically high extracellular magnesium concentrations on the proliferation of chondrocytes was only tested on chondrocytes cultured under monolayer cultures and not under any other culture conditions. Since the prior art at the effective filing date of the present application does not provide such guidance for the above mentioned issues, it is incumbent upon the present application to do so.

Furthermore, the physiological art is recognized as unpredictable (MPEP 2164.03). As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues set forth above, the state of the relevant physiological art on the generation of chondrons at unphysiologically high extracellular concentrations of magnesium, and the breadth of the claims, it would have required undue

experimentation for one skilled in the art to make and use the instant broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-10, 14 and 18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. ***This is a new ground of rejection.***

In independent claim 1 and its dependent claims, it is unclear what is encompassed by the limitation "wherein said second unphysiologically high extracellular concentration **is used during chondrocyte differentiation**". This is because cultivating **chondrocytes are already differentiated cells**; thus it is unclear at which chondrocyte differentiation stage the second unphysiologically high extracellular concentration of Mg is used as recited by the instant claims. It appears that the second cultivating step of the method in claim 1 misses an essential element for “differentiating” the already differentiated chondrocytes so that a second unphysiologically high extracellular concentration of Mg can be used. Clarification is requested because the metes and bounds of the claims are not clearly determined.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

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